borders in the BIBAC were generated by *Pfu* polymerase PCR using primers which hybridize to cosmid pVK232 (Knauf and Nester 1982). The plasmid pVK232 contains the entire TL-DNA of TiA6 (de Vos et al. 1981; Barker et al. 1983; Gielen et al. 1984). A synthetic polylinker connects the border sequences, and is designed to provide unique cloning sites adjacent to the right and left border sequences to facilitate the introduction of selectable markers. These are designated MSR (marker site right) and MSL (marker site left) in Figure 1. Very large segments of DNA between the borders can be transferred into certain host cells (Miranda et al., 1992). The PCR generated borders and the polylinker were initially cloned into pUC19.

[0052] As a result, the right and left borders and the polylinker are carried on a *Sal* I fragment in pCH7. This was done to facilitate the subsequent cloning of this fragment into the *Sal* I site of mini-F pMBO131.

[0053] The *sacB* gene was cloned into pCH7 by transforming a *pcnB* strain of *E. coli* and incubation of the transformants at 30°C. If a pUC type plasmid is propagated at 30°C, then its copy number is reduced from 80 to 20 copies per cell (Lin-Chao et al. 1992). The *pcnB* (plasmid copy number) mutation reduces the copy number of ColE1 type plasmids from about 20 copies per cell, to about 1 copy per cell (Lopilato et al. 1986). These conditions were used to obtain the desired construct, pCH10. When the *Sal* I fragment from pCH10 was introduced into the mini-F to make pCH13, positive clones were easily identified by screening for the *sacB* marker. The oriT/Ri origin fragment was then introduced into a unique *Hpa* I site which is 35 nucleotides from the *Sal* I edge of the mini-F to make pCH16. Preliminary experiments indicated that chloramphenicol is not a useful marker for some strains of *Agrobacterium*, so a kanamycin resistance gene cassette (Smith and Crouse 1989) was cloned into the *Pvu* II site of the chloramphenicol gene of the mini-F to complete the backbone of the BIBAC.

[0054] The GUS-NPTII construct (Datla et al. 1991) is a bifunctional fusion peptide between *E. coli* β-glucuronidase (GUS) and neomycin phosphotransferase II (NPTII). This GUS-NPTII construct is carried on an *Eco* RI-*Hin*d III fragment. The fragment was treated with Klenow and ligated into the *Srf* I site at the MSL site to create BIBAC1. Though this GUS-NPTII construct is effective as a selectable and screenable marker in plant cells, it will be less useful as a marker in other hosts of the BIBAC, such as microorganisms and animal cells. The GUS-NPTII construct can be excised and replaced with an aminoglycoside 3-phosphotransferase (AGP, kanamycin-resistance) gene which encodes resistance to the drug G418. This drug, in conjunction with an AGP gene under the control of appropriate gene regulatory sequences, has been effective as a selectable marker in diverse types of cells, including yeast, other fungi, insects, and mammalian cells.

[0055] If two selectable markers are desired in a BIBAC vector, the promoters and transcription termination sequences of the two constructs should be nonhomologous. This is to prevent any sequence redundancy within the vector which might reduce its stability. In addition, any pairs of selectable markers should be oriented in the BIBAC so that they are not convergently transcribed, as this configuration may impair transgene expression (Jones et al. 1992). When a large segment of DNA is inserted such that the selectable markers are separated by many kb of DNA, this is not likely to be a problem. However, control experiments using the vector alone might be compromised.

[0056] Different selectable markers can be incorporated into the BIBAC for maintenance of the plasmid in the host cell of interest. For example, BIBAC3 carries the HYG construct at the MSL. The hygromycin phosphotransferase (HYG) construct provides resistance to hygromycin (Becker, et al. 1992). The HYG construct in BIBAC3 has promoter and terminator regions

appropriate for selection in plant cells. These could be replaced with promoters and terminators for other host cells such as fungi or animal cells. Any other selectable marker can be readily introduced at one of the unique polylinker sites. Of course, the selectable marker must not have a *Bam* HI site, so that the library cloning site will still be unique.

EXAMPLE 2

Testing of the BIBAC Vector

[0057] A physical map of BIBAC1 has been established, and the vector alone (without any DNA insert) functions as expected. It replicates in *E. coli* and *A. tumefaciens*. Tomato and yeast heterologous DNA have been inserted into the BamHI site of the BIBAC vector. Each resulting clone (which includes the vector and the heterologous DNA) was then introduced into *Escherichia coli* strain DH10B by electroporation. The *E. coli* strain DH10B has been widely used for construction of genomic libraries, and stability is not expected to be a problem for the majority of BIBAC clones. The DH10B strain contains *recA1* which increases the stability of the inserts, as well as *mcr*A, *mcr*B, *mcr*C, and *mrr*, which in combination prevent the restriction of DNA which contains methylated cytosine and adenine residues. That is, it should not be a problem to clone even heavily methylated genomic DNA using this strain.

[0058] A triparental mating was then performed with the resulting *Escherichia coli*, an *Escherichia coli* containing a helper plasmid pRK2073 (which carries resistance to spectinomycin (Leong et al. 1982)), and *Agrobacterium tumefaciens*. The vector was successfully transferred and was stable in *Agrobacterium tumefaciens.* These E. coli and Agrobacterium strains carrying the BIBAC with inserts of yeast and plant DNA can be screened by standard methods to detect chemicals and other products produced by the bacteria as a result